EDITORIAL

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Getting to the Source: Dendritic Cells as Therapeutic Reagents for the Treatment of Patients With Cancer

It seems that every generation has a new set of challenges and insights in understanding the immune response and how it works, then developing the means for modifying it. I do recall, as a medical student 25 years ago, entering the operating room and having the general surgeon, struggling with a colon cancer resection, turn to me and say, "What is all this about T cells?" At that time, immunology had enjoyed more than a century of intense study and investigation of antibodies and the remarkable properties of the phagocyte as championed by Ehrlich and Metchnikoff, respectively, but little was known about the cellular immune response, mediated by T cells.[1] This was rectified in an extraordinary and exuberant explosion of information about the important role of T cells in mediating the so-called cellular immune response (as opposed to humoral- or antibody- mediated immunity). We now know much about the targets of T cells, the role of individual class I and class II molecules in presenting peptides to T cells, the generation of T-cell diversity, the education and maturation of T cells within the thymus from precursors derived in the bone marrow, and so on. What remained obscure for many years, and was only susceptible to study in the last 10 to 15 years, was how immune responses were initiated by a subpopulation of cells in the peripheral blood, probably representing $< 1\%$ of all cells, known as dendritic cells (DCs).

Dendritic cells, originally identified by Steinman and colleagues,[2] represent the pacemakers of the immune response, the source of antigenic peptides and proteins presented to T cells and B cells, and they are now widely recognized as the professional antigen-presenting cells. Generated in large numbers in the bone marrow every day, they course through the blood stream, migrate into tissues, and after various residency times ranging from hours to weeks, complete their circuitous journey by responding to local cytokines, picking up antigen at the sites of inflammation, carrying it to the resident lymph nodes, and selecting those few T cells and B cells that are capable of responding to the antigen in an optimum fashion. After delivering their message, the DCs, no longer necessary for the acute response to antigen, presumably undergo apoptotic death and are eliminated.

Among of the reasons these cells have been thrust into the mainstream of modern immunologic thought are their extraordinarily potent ability to stimulate primary immune responses and the remarkable recent advances in the ability to culture them from CD34+ bone marrow hematopoietic precursors and from peripheral blood-derived adherent macrophages.[3–8] Although it was once thought that these cells were nonphagocytic, it is clear, particularly after culture with the cytokines GM-CSF and interleukin (IL)-4, that they are actively phagocytic and take in up to 2400 fL of fluid every hour—the equivalent of their own cell volume.

Given the fact that the adaptive immune response represented by B cells and T cells is dependent on the presence of DCs to elicit an immune response during the initial periods of inflammation, their importance has become clear. Those interested in infectious diseases, autoimmunity, transplantation, and cancer[9] should pay attention to these cells. There is some evidence that these cells may be involved with tolerance generation[10,11] and with eliciting effective immune responses to tumor. Clinical trials applying these cells have been started at a number of institutions including Duke University, the University of Southern California, and the University of Pittsburgh; the initial trials in patients with B-cell lymphomas were reported by Engleman and Levy at Stanford University more than a year ago.[12]

Where do DCs fit in? It appears now that the so-called follicular dendritic cell lying resident in follicles within the cortex of the lymph node actually are derived from

Table 1. RELATIONSHIP BETWEEN DENDRITIC CELL INFILTRATION OF HEAD AND NECK CANCERS/ORAL CAVITY CANCERS AND PROGNOSIS

Tumor	Reference	Dendritic Cell Infiltration
Larynx	Orl 53:349, 1991	Present in tumors
Nasopharyngeal	Laryngoscope 101:487, 1991	Markedly improved prognosis*
Larynx	Chin J Otorhino 27:297, 1992	Markedly improved prognosis*
Oral	J Oral Path&Med 21:100, 1992	Less with smokeless tobacco
Oral	J Cut Pathol 19:398, 1992	Less in tumors
Head and neck	Cancer Immther 36:108, 1993	PBMC DC less functional
Larynx	In Vivo 8:229, 1993	Improved prognosis*
Head and neck	Cancer Immther 38:31, 1994	Present in some tumors
Oropharynx	In Vivo 8:543, 1994	Less in tumors*
Oral squamous	J Oral Path&Med 24:61, 1995	No relationship with prognosis

* Indicates statistically significant difference when compared to either normal or less DC's.

nonhematopoietic precursors and are the sink for antigen to allow long-lived immune stimulation of B cells. The DCs that have captured most people's attention, however, are derived from hematopoietic precursors. They are identified[2,13,14] as most closely related to macrophages (myeloid DCs) and should be distinguished from a second, more recently identified cell population closely related to T cells, natural killer cells, and B cells (lymphoid DCs). The lymphoid DC's role in regulating immunity still must be clarified.[15,16] Perhaps one of the most remarkable recent observations has been the finding that one of the stem cell factors, Flt 3 ligand, not only causes dramatic increases in the number of myeloid and lymphoid DCs in the spleen and peripheral tissues, it also mediates important antitumor effects in murine tumor models.[17,18] This suggests that just increasing the number of antigen-presenting cells may be sufficient to augment immunity.

What about the role of DCs in cancer biology? Clinical pathologists[19] recognize that there is a direct relationship between the presence of DCs within lung tumors, head and neck tumors, and other kinds of tumors, and prognosis (Table 1 and Table 2), (*i.e.* the presence of more DCs is associated with a better prognosis). We recently confirmed these findings in tumors of the oral tongue.[20] How do we understand this observation? Are DCs there to pick up tumor antigen? Are they there to provide an environment hospitable for recruited T cells? We first must examine what seems to be a series of evolving precepts within tumor immunology. The biologic therapist has four primary goals to treat cancer: 1) To elicit an immune, largely T-cell response to tumor. Dendritic cells are notably efficient and perhaps necessary to recruit, select, and expand T cells with antigen specificity, which can, in turn, be exported to tumor sites as immune effectors capable of killing targets and releasing cytokines. 2) To allow T cells to migrate across endothelial barriers. This has been studied in a limited way by tumor immunologists, but it is clear that means to drive T cells into tissues, particularly at the sites of tumors that do not elicit effective inflammatory responses, must be examined critically. Whether DCs play a role in this process is unclear. 3) To maintain a state of effectiveness of T cells at the tumor site. Perhaps one of the most remarkable finds this year has been that numerous tumor cells, including melanoma, colorectal cancer, hepatoma, and lung cancer,[21–26] express a molecule on their cell surfaces that is a counter receptor known as Fas and is expressed ubiquitously on target cells, especially on activated T-cells entering the tumor site. In susceptible T cells, this causes the induction of apoptosis. Thus, we have the seemingly paradoxical situation of tumors killing T cells instead of T cells killing tumors. For that reason, means to try to prevent premature T-cell apoptotic death and the engagement of so-called T-cell futile cycles is another goal of the biologic therapist. Dendritic cells, by virtue of their expression of so-called costimulatory molecules, such as CD80 (B7.1) and CD86 (B7.2), and several important cytokines, including alpha-interferon and interleukin-12,[27–29] may be uniquely capable of preventing premature T-cell death and may become the mediators of T-cell survival. 4) To maintain a state of long-lived immunity, similar to what is observed after viral infection. Clearly, DCs are capable of providing the appropriate stimulus under many circumstances to generate and to maintain memory.[30] Thus, for many reasons, the administration of DCs appears to be a particularly good means of eliciting an effective immune response. In addition, presumably because they can be given at numbers in clinical trials far fewer than what is necessary for T cells (the final effectors in many tumor models), they have considerable charm for the biologic therapist.

The major concern of DC biologists is how to use these cells in therapy. One question has to do with the source

Table 2. RELATIONSHIP BETWEEN DENDRITIC CELL INFILTRATION AND PROGNOSIS IN MALIGNANCY

Tumor	Reference	Dendritic Cell Infiltration
Basal cell	Brit J Derm 130:273, 1994	Less in tumors*
Basal cell	Brit Derm 127:575, 1992	? Improved
Breast	J Pathol 163:25, 1991	? Improved prognosis
Bronchoalveolar	Eur J Ca 28A:1365, 1992	No effect
Cervix	Am J Clin Path 99:200, 1993	Less in HPV+ tumors
Cervix	Cancer 70:2839, 1992	Improved*
Cervix, stage III	In Vivo 7:257, 1993	Marked improved prognosis*
Cervix/penile	J Urol 147:1268, 1992	Less with HPV infection
Cervix/HIV	Gynec Oncol 48:210, 1993	Less in AIDS
Esophageal	Virchows Arch 61:409, 1992	Marked improved prognosis
Esophageal	In Vivo 7:239, 1993	Direct relationship to Grade
Gastric	Int Surg 77:238, 1992	Marked improved prognosis*
Gastric	Cancer 75:1478, 1995	More in tumor draining LNs
Gastric, stage III	In Vivo 7:233, 1993	Marked improved prognosis*
Hodgkins disease	Am J Cl Path 101:761, 1994	FDC improve prognosis*
Lung	Pathology 25:338, 1993	Marked improved prognosis*
Lung	J Clin Invest 91:566, 1993	Related GM-CSF production
Melanoma	J Invest Derm 100:269, 1993	Inverse with tumor thickness
Mycosis fungoides/Sezary Syndrome	In Vivo 7:277, 1993	Marked improved prognosis*
Prostate	Prostate 19:73, 1991	Improved prognosis
Skin tumors	Arch Derm 131:187, 1995	Less in tumors
Thyroid (papillary)	Zent f Chir 117:603, 1992	No effect

* Indicates statistically significant difference when compared to either normal or less DCs.

of cells. The two predominant sources that clinicians and scientists have contemplated are CD34+ precursors obtained from bone marrow or G-CSF-mobilized stem cells obtained from peripheral blood. An alternative approach is to perform a leukophoresis to obtain sufficient macrophages, which can be isolated by adherence and matured by brief 5 to 7 day cultures in the cytokines GM-CSF and IL-4. The major issue addressed in the article by Morse and colleagues (pp. 6–16) has to do with the use of tumor necrosis factor-α to mature these cells into cells expressing one of the markers of mature DCs, CD83.[31,32] In some ways, this may represent the most stimulatory cell in alloreactivity. Unfortunately, in our murine tumor models,[33–36] these cells appear to be incapable of eliciting antitumor responses, whereas GM-CSF/IL-4-cultured cells elicit therapeutic effects against even late (28-day-old) tumors. Perhaps this is in part because they have lost their phagocytic capability or because of problems related to trafficking to appropriate secondary lymphoid sites. Clearly, phenotypic or allostimulatory capacity—each a hallmark of mature DCs—cannot be used alone to distinguish effective versus ineffective cells for use in clinical trials. Furthermore, although TNF is thought to be the proximal signal promoting tissue DC migration to secondary lymphoid sites, including lymph nodes, it also can limit generation of reactive T cells.[37]

There are other novel markers that can be used to distinguish DCs from other cells. These include CD1, S100 staining, high expression of the major histocompatibility complex class II molecules, and, most recently, expression of the p55 actin-bundling protein, a cytosolic marker that was found within Epstein-Barr virus transformed B cells, within follicular and nonfollicular DCs, and within the Reed-Sternberg cells in all but the lymphocyte-predominant forms of Hodgkin's disease.[38] Dendritic cell purists would suggest that there may be major functional differences between DCs derived from macrophage precursors, also now known as dendriphages, and those derived from CD34+ bone marrow precursors.[39] This issue, unfortunately, can be resolved only in a prospective randomized trial, which we plan to initiate within the next few months.

One other issue worth addressing has to do not only with the role of cytokines in DC maturation, as discussed by Morse et al., but also with defining which cytokines are made by DCs. Interestingly, DCs serve as a major source of two cytokines, one with an older history, interferon-alpha (IFN-α), and the more recently defined interleukin 12 (IL-12). Dendritic cells also produce MIP1γ, IL-1, IL-6, and IL-15, all of which are important in the elicitation of a primary immune response.[32,40] Interleukin-12 production is critical for the promotion of a cellular

immune response. Its production, interestingly, appears to be inhibited by various tumor-derived substances, including nitric oxide, PGE2, IL-10, IFN-α itself, the p40 homodimer of IL-12, and the p40 analog EBI-3.[41] Transforming growth factor-β appears to promote the maturation of DCs,[42] which is puzzling because it is largely regarded as an immunosuppressive cytokine.

The final issue that must be addressed is how best to get tumor antigen into DCs so that an effective immune response can be elicited. The approach championed by our group[33–36] and by others[44] has been to use synthetic peptides derived from tumor antigen protein precursors, such as the melanoma-associated antigens MART 1/Melan A, tyrosinase, or gp100, and to use the protein or genes designed to modify DCs to express these antigens.[44,45] Murine models suggest that some peptides used alone can induce tolerance unless they are presented by mature DCs.[46] Alternatively, when the antigens are unknown, various approaches have been described. These range from using tumor lysates, messenger RNA derived from the tumor itself, stripped peptides (acid eluted from MHC class I or Class II molecules on the tumor cell surface), or even fusing the tumor with DCs. All seem cumbersome, especially given the small size of some human tumors when they are resected. An alternative strategy, and one that we are exploring, is to put DCs into tumors instead of putting tumor antigens into DCs. The tumor environment appears to be immunoinhibitory,[47] and means first to stimulate DCs using cytokines or bacterial antigen[48] may be necessary. This is an area of considerable interest; others are exploring it, too.

In summary, DCs may be the Holy Grail of the biologic therapist, because of their extraordinary ability to elicit and to maintain an effective T-cell response and their relative abundance and ease of culture from peripheral blood and bone marrow. The next few years should see an explosion of clinical protocols and a resolution of many of the unanswered questions related to their biology. It is likely that during this period, effective therapeutic clinical approaches will evolve. Rather than removing a tumor and shipping it for formalin fixation by a pathologist, some day we may be able to deliver DCs to the site for a few days and then resect the residual tumor for further subsequent DC delivery of tumor antigens. For those interested in this approach, the 5th International Symposium on Dendritic Cells in Fundamental and Clinical Immunology will be held in Pittsburgh, Pennsylvania from September 24 to 28, 1998, and you are certainly encouraged to contact us for additional information!

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